Predictable & precise & protective – a new CRISPR/Cas tool to insert large cargo

Problem
CRISPR/Cas9-mediated gene integration often is based on HDR- or NHEJ-mediated mechanisms with negative effects:
- unpredictable DNA repair
- lack of precision
- genome damage inflicted

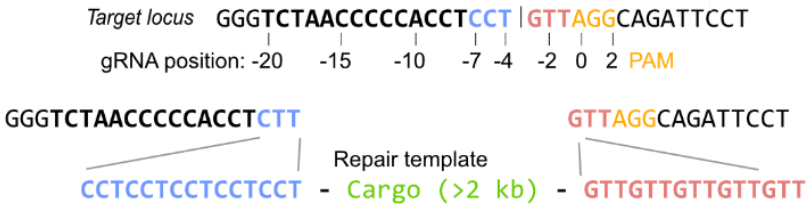
Solution
New methodology uses AI-optimized repeats of 3nt microhomologies (trimologies) and maximizes gene **knock-in** → increases the fidelity of **large** gene insertions at the target site, while maintaining efficient editing.

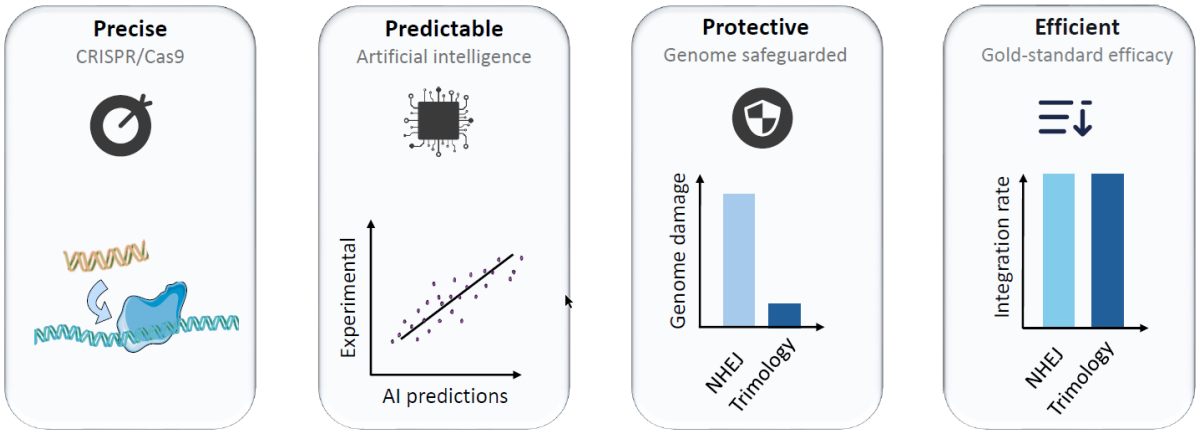
Background

Integrating large cargo presents persistent challenges, despite leveraging the CRISPR/Cas9 system. The outcomes of DNA repair at the junctures between the genome and insertional cargo remain unpredictable. This unpredictability often results in the loss of genetic information, both within the genome and the cargo itself. This loss is undeniably detrimental and highlights the need for improved precision and reliability.

Trimology integration

Knock-in of large cargo – 2.5 kb validated
(*ie.* therapeutic cargo, chimeric antigen receptors)





Verified for diverse targets
using distinct delivery modalities

Cell lines	Embryos	Adult mice
LNP transfection Electroporation	Micro-injections	Viral delivery

Invention	A methodology for CRISPR/Cas9 ***knock-in*** using ***repair*** donors with small repeats of microhomologies (“trimologies”) flanking the transgene cassette to obtain predictable DNA repair outcomes at the transgene-genome boundaries when performing CRISPR/Cas9.
Use	Targeted single-copy integration of large cargo (2.5 kb) into embryos, adult tissues and human cell lines for the developing of new models or gene therapies. Genomic integration is also feasible in fully differentiated, non-proliferating cells (i.e. adult neurons).
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Patent Status	Patent filed
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